Estimation of Nutritive Value, Organoleptic Properties and Consumer Acceptability of Fermented Cereal Gruel (OGI)

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Abstract: A gruel, locally called Ogi in Nigeria and prepared by bacterial fermentation from cereals such as maize (Zea mays) and (Sorghum bicolor) of the Poaceae Gramineae family remain a major source of calorie, nutrient and probiotic factors in human diets in many West African countries including Nigeria. Therefore, knowledge on nutritive, organoleptic and safety values of OGI is hugely essential to justify its dietary utilization and fortification as weaning diets and staples and the need for improvement in cereal fermentation. In this study, we determined the indices of nutrition, palatability and preservation in Ogi made from cultivars of maize and sorghum in Nigeria using Lactobacillus pentosus and L. acidophilus as starter cultures during fermentation. Eleven cultivars of corn and sorghum sold in Nigerian markets were used for the preparation of fermented gruels using L. pentosus and L. acidophilus as single and mixed starter cultures. Gruels from these cultivars were also prepared under uncontrolled fermentation conditions and used as control. These gruels were compared by proximate composition, probiotic and organoleptic values using standard chemical, chromatographic and spectrophotometric methods. The biochemical analysis of the fermented samples showed the concentration of acid, acetoin and diacetyl to increase in comparison to the controls indicating improved organoleptic properties. All samples showed increased levels of reducing sugars, proteins, and amino acids. Essential amino acids, lysine, isoleucine, and arginine were elicited in all fermented samples, showing desirable nutritional status. All these fermented samples exhibited some degree of inhibition on all the test bacterial cultures. The mixed culture fermented samples had higher values than the single culture fermented samples for all parameters tested indicating higher efficacy.

Key words: Arginine, isoleucine, lactobacillus, lysine

INTRODUCTION

Cereal based foods are a major source of inexpensive dietary energy and nutrients in developing countries. They are processed in various ways, the most popular being through fermentation. This process is known to suit the socio-economic framework of developing countries as an affordable technology for the preservation and improvement of high carbohydrate based foods (FAO/WHO, 1997). Fermentation has remained an important The fermentation of cereals and cereal-based products in the traditional way depends largely on chance inoculation, involving mixed cultures of bacteria, yeasts or both. The common fermenting bacteria are the species of Leuconostoc, Lactobacillus, Streptococcus, Pediococcus, Micrococcus and Bacillus. Most bacterial fermentations are usually Lactic acid fermentations, while the yeasts bring about alcoholic fermentation. In addition, some mold species such as Aspergillus, Penicillium, Fusarium and Cladosporium may be involved in certain products. Lactic acid bacteria have been reported as the major fermenting organisms in cereal fermentation. The common use of Lactic Acid Bacteria (LAB) for food fermentation in Africa is most probably due to the beneficial role of preservation, enhanced nutritional value, detoxification, rendering inedible foods edible, and production of variety in flavour intrinsic to the usage. Members of the genus Lactobacillus constitute a most important member within the LAB group and one of the most useful in food industry (Davidson and Hoover, 1998; Olasupo et al., 1997). Though the spontaneous process involving chance inoculation by natural microflora is the cheapest method, used in preparation, its contamination is a major cause of diarrhoeal diseases and associated malnutrition especially in children (Olukoya et al., 1994; Mensah et al., 1990; Black et al., 1982). Microbial fermentation has been reported to aid recolonisation in malnourished children whose microfloral balance has been altered due to acute diarrhoea (Oyetayo et al., 2003). There is a need for standardization of fermentation conditions so as to obtain a product with acceptable sensory properties and improved nutritional quality. The use of selected starter cultures for fermentation during processing of foods has been found to improve shelf life, nutritional status and to promote safety (Cook, 1994; Steinkraus, 1996).
Aim: The production of a cereal-fermented gruel (OGI), a popular weaning food in the West African zone was carried out using mixed and single cultures of Lactobacillus pentosus and Lactobacillus acidophilus as starter cultures. The nutritive value, the metabolic products, and sensory evaluation were examined to determine its suitability as a nutritionally rich infant formula with acceptable organoleptic and probiotic properties.

MATERIALS AND METHODS

Collection of samples: Eleven varieties of maize (Zea mays L.) used were collected from Maize Research Laboratory of the International Institute of Tropical Agriculture, (I.I.T.A.) Ibadan while the sorghum varieties (Sorghum vulgare) were purchased from Mile 12 Retail Market, in the Lagos metropolis and authenticated by the Department of Botany, Lagos State University, Ojo. The maize varieties are: TZPB-SR, TZB-SR-SE, EV8363-SR,QPM, TZSR-W-1, TZB-SR, 8321-21, SUWAN-1-SR,QPM, EV8766-SR-Y,QPM, TZSR-Y-1, 8321-18, EV8762-SR, and designated 1-11, respectively. The red, yellow, and white sorghum varieties were designated 12-14, respectively. These cultivars are currently sold in the various local markets in Nigeria.

Production of OGI and proximate analysis: The fourteen varieties of maize and sorghum seeds (i.e., cultivars) used were individually processed and fermented to make OGI. Briefly 500 g of each cultivar (dirt and shaft free) were steeped in 1.5 L of sterile water containing 5% of sodium metabisulphite for 24 h to prevent microbial contaminants and to surface-sterilize the grains. The seeds were then washed three times repeatedly with sterile distilled water, drained, blotted dry using sterile filter papers, re-steeped in 2 L of sterile distilled water for further two days and finally wet-milled under sterile conditions. The resulting dough (i.e., wet-milled cereal) was then sieved using a sterile wire mesh (0.2mm. pore size) to obtain fine slurry (filtrate), collected in a sterile conical flask, leaving behind a pomace on the mesh. Each slurry was divided into three portions, followed by fermentation for 3 days at 30°C±2ºC via direct inoculation 06 cfu/mL of mixed and single cultures of Lactobacillus pentosus NG 578 and L. acidophilus NG75 strains in the laboratory. For the control, 500 g portion of each cereal cultivar was fermented uncontrolled at 30°C±2ºC without direct inoculation with the starter cultures (i.e., chance fermentation) as previously described by Odunfa (1985). The fermented samples were analyzed for their nutritional contents such as total protein and carbohydrates, organoleptic indices, including the levels of organic acid, acetoin, and diacetyl and sensory values as described in Fig. 1.

Determination of total protein: The Micro-Kjeldahl method for protein determination was employed (Ma and Zuazaga, 1942).

Amino acid determination: Rosen (1957) method which is a modified Ninhydrin method was used in determining the amino acid profile of the cereal gruels. Approximately 2 g of slurry was suspended in 20 mL of phosphate buffer (pH 7.0) in a 250 mL beaker. The suspension was centrifuged with Refrigerated Centrifuge Model GL 18B at 3000 rpm for 10 min and the supernatant poured into a separating funnel, and shaken with 10 mL petroleum ether to remove the organic pigments. The top phase was discarded and the aqueous phase which contained protein and amino acids was retained. The protein was precipitated from the aqueous phase by adding 5 mL of 10% (v/v) trichloroacetic acid (TCA) to 5 mL of the extract. The mixture was shaken and kept in the freezer for 10 min. The precipitate formed was removed by centrifuging and the filtrate was used for total amino acid profile determination using Thin Layer Chromatography (TLC).

Chromatographic separation of amino acids: The amino acid contents of the extracts were separated by thin layer chromatography using the method of Mikes and Chalmers (1989).

The thin layer plate was dried at 110°C for 10 min before use as TLC plates tend to pick up moisture from the air and this can considerably reduce their ability to adsorb the sample. Aliquots of 50 μL of each filtrate were spotted on thin layer glass plates along with 20 μL of reference standard amino acids. The standard amino acids (0.1% w/v) were isoleucine, lysine, tryptophan and methionine. One dimensional ascending chromatography
was used. The solvent system employed for the separation was n-butanol: glacial acetic acid: water at a ratio of 4:1:2 (v/v/v) and separation was carried out for 3 h. The chromatograms were air-dried and the amino acids located by spraying with 0.2% (w/v) ninhydrin in ethanol. These were allowed to air-dry and then oven-dried at 100°C for 5 min for the spots to develop. Identification of the separated amino acids was done using the reference standard.

The quantitative estimation of the amino acids was done using the colorimetric method (Rosen, 1957). The supernatant of each fermented gruel was prepared and used to determine the concentration of each amino acid present. Each supernatant was made up to 2 mL with distilled water and 1 mL ninhydrin solution was added. The absorbance taken at 570 nm was read using a spectrophotometer (Spectronic Unicam Type Helios Gamma NC 9423 UNG Model). Absorbance readings were taken in triplicate. The control test tube contained 2 mL. distilled water and 1 mL ninhydrin solution. The concentration of each amino acid was extrapolated from a standard curve previously prepared.

**Determination of reducing sugar:** This was carried out using the Nelson-Somogyi procedure (Somogyi, 1952).

**pH determination:** The pH of each sample was determined using a pH meter (Metrohm 780pH meter). This was determined for the wet slurry as well as for the flour.

- For the wet samples 10 mL of slurry was mixed thoroughly and allowed to sediment. Five mL of the supernatant water was taken and the pH determined.
- To 2 g flour was added 10 mL of sterile distilled water. This mixture was thoroughly mixed using an autovortex mixer to enhance homogeneity and then filtered using Whatman No.1 filter paper. The pH of the filtrate was taken.

**Determination of titratable acidity:** Five mL of filtrate from 1(ii) was added 3 drops of phenolphthalein indicator. Then 1mL of 0.1M NaOH was made up to 20 mL and 5 mL of this was then made up to 20 mL with distilled water. The filtrate-phenolphthalein solution was then titrated with the new concentration of 0.1M NaOH. The colour change, titre value, and volume of filtrate used were noted.

**Detection and quantitative estimation of acetoin and diacetyl:** The colorimetric method of Westerfield (1945) was used to determine the concentration of acetoin and diacetyl.

**Sensory evaluation:** Sensory evaluation of the fermented cereal gruels was carried out by use of 10 untrained taste panellists in a special room prepared for the purpose at the University of Lagos (Ebuehi et al., 2004). They were instructed to taste the fermented cereal samples and to rinse their mouth after each taste. They were requested to express their feelings about the samples by scoring the following attributes: appearance, texture, taste, aroma and overall acceptability. Questionnaires were distributed to each of the panellists and sensory scores were based on a 9-point hedonic scale, where 1 is dislike extremely and 9 is like extremely (Watt et al., 1989). The responses of the panellists with regard to their preference for the samples were statistically analyzed. The responses of the panellists with regard to their preference for the samples were statistically analyzed.

**RESULTS**

The nutritional status showed increase in concentration of proteins and amino acids in all samples with the mixed culture samples having the highest values. Total protein value was highest in those fermented with mixed culture having an average percentage value of 13.13±4.33 on day 3, followed by *L. pentosus*-fermented with a value of 10.97±4.37, then *L. acidophilus*-fermented ones with a value of 9.62±4.50 and the locally-fermented having the lowest value of 7.78±2.15 (Fig. 2).

Table 1 and 2 show the result of quantitation of the amino acids present in all the fermented cereal gruels. Levels of lysine ranged from 195.7-720.7 mg/100 g in the *Lactobacillus*-fermented cereal gruels compared to 58.4-95.2 mg/100 g in the fermented cereal gruels produced by spontaneous inoculation (p<0.05) and methionine content ranged from 260.7-652.4 mg/100 g in *Lactobacillus*-fermented cereal gruels to 65.2-143.8 mg/100 g in the control. The values of isoleucine and tryptophan also followed the same pattern of 201.6-631.8 mg/100 g and

![Fig. 2: Variations in average total protein content with days of fermentation in four varieties of cereals, A = cereal fermented with *L. acidophilus*; B = cereal fermented with *L. Pentous*; C = cereal fermented with mixed culture; D = control. *p<0.05 (compared to the)](image-url)
Table 1: Analysis of fermented cereal cultivar gruels (OGI) for limiting amino acids: Lysine and Methionine

<table>
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<tr>
<th>Cultivar</th>
<th>A</th>
<th>B</th>
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<th>A</th>
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<td>422.6±27.8</td>
<td>415.3±26.3</td>
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Table 2: Analysis of fermented cereal cultivar gruels (OGI) for limiting amino acids: Isoleucine and Tryptophan

<table>
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Data is presented as mean ± standard deviation from the mean (SD) of three determinations and analyzed by student’s t-test (2-tail) Cultivars of maize (1-11) as obtained from ITTA. Red, yellow and white sorghum cultivars (12-14) were bought from the market; A: L. acidophilus; B: L. pentosus; C: Mixed culture; D: Control; p<0.05 was considered significant; a: p<0.05 (D vs. A or B or C); b: p<0.05 (A vs. B or C); c: p<0.05 (B vs. C); d: p<0.05 (A vs. B); e: p<0.05 (A vs. C); f: p<0.05 (B vs. A or C)

Further analysis revealed significant disparity. When compared with the gruels fermented by chance inoculation respectively.

Further analysis revealed significant disparity (p<0.05) in the contents of these amino acids with respect to the inoculating specie and the cereal cultivar tested. Lactobacillus acidophilus elicited the highest contents of lysine in 10 of 14 cultivars, methionine and tryptophan in 12 of 14, and isoleucine in 9 of 14, when compared with L. pentosus. When compared with the gruels fermented by mixed culture of Lactobacillus, L. acidophilus elicited highest lysine and isoleucine contents in only 1 of 14 cultivars, 8 of 14 in methionine, and 7of 14 in tryptophan. L. pentosus had the highest values of tryptophan in 2 of 14 when compared with other fermented gruels. These two were the sorghum cultivars. L. acidophilus seemed to have elicited amino acid in the cereals more than L. pentosus.

The amino acid profile using thin layer chromatography revealed the presence of lysine, methionine, and tryptophan among the essential amino acids and isoleucine among the semi essential amino acids in all the fermented cereal gruels. However, arginine, a non-essential amino acid occurred in some cereal gruels (Fig 3a - d).
Fig. 3a: Amino acid paper chromatogram of some spontaneously-fermented cereal gruels. Try: Tryptophan, Iso: Isoleucine, Ly: Lysine, 3: EV8363-SRQPM, 7: SUWAN-1SRQPM, 12: Red Sorghum, 13: Yellow sorghum, 6: 8321-21

Fig. 3b: Amino acid paper chromatogram of some L. acidophilus-fermented cereal gruels. Try – Tryptophan, Iso: Isoleucine, Ly: Lysine, 3: EV8363-SRQPM, 7: SUWAN-1SRQPM, 12: Red Sorghum, 13: Yellow sorghum

Fig. 3c: Amino acid paper chromatogram of some L. pentosus-fermented cereal gruels. Try: Tryptophan, Iso: Isoleucine, Ly: Lysine, 3: EV8363-SRQPM, 7: SUWAN-1SRQPM, 12: Red Sorghum, 13: Yellow sorghum

Fig. 3d: Amino acid paper chromatogram of some mixed culture-fermented cereal gruels. Try: Tryptophan, Iso: Isoleucine, Ly: Lysine, 3: EV8363-SRQPM, 7: SUWAN-1SRQPM, 12: Red Sorghum, 13: Yellow sorghum

Fig. 4: Concentration of reducing sugar in different fermented cereal gruels relative to fermentation time. Data given as mean ±SD

Fig. 5: Mean total acetoin concentration in fermented cereal gruels

The Nelson-Somogyi method used in determining the reducing sugar amount revealed L. pentosus-fermented samples to have an average of 44.9±31.26 mg/mL, L. acidophilus-fermented samples had 46.44±31.77 mg/mL and those fermented by mixed culture 49.81±32.63 mg/mL, while the spontaneously-fermented had the lowest value of 29.58± 8.03 mg/mL (Fig. 4).
Fig. 6: Mean total diacetyl contents of fermented cereal gruels

Fig. 7: Sensory Evaluation of Ferment Cereal GruelsEach bar represents mean % score of attributes. Vertical lines on bars indicate standard error of mean (SEM). *p<0.05 compared to spontaneously fermented cereal gruel; Bp<0.05(Mixed culture vs. monocoltire fermented cereal gruel)

Fig. 8: Mean pH of fermented cereal gruels

The concentration of acetoin, diacetyl, and acid increased in starter culture samples in comparison to the controls indicated improved organoleptic properties. Flavour-enhancing diacetyl and acetoin levels in the products were significantly increased during fermentation using the starter culture strains individually but was exceptionally higher (p>0.05) when mixed culture was used. The amount of acetoin (mg) produced in cereals locally fermented was found to be lowest with an average of (0.05±0.027) on day 1 to (1.48±0.48) on day 3, while the mixed culture-fermented had the highest values of (0.10±0.027) to (9.42±3.24) (Fig. 5).

The diacetyl amount followed the same pattern. The amount of diacetyl (mg) produced in cereals locally fermented was found to be lowest with an average of 1.16±0.35 on day 3, followed by *L. pentosus*-fermented cereals 1.51±0.69, followed by *L. acidophilus* fermented cereals with average value of 1.92±0.71 and highest in those fermented with the mixed culture with value of 3.50±0.50 (Fig. 6).

Organoleptic assessment of the cereal gruels revealed variations in scores for taste, aroma, colour, texture, and overall acceptability. Generally, gruels made from *Lactobacillus* fermentation elicited significantly (p<0.05) higher organoleptic scores for each of the sensory attributes investigated compared to the spontaneously fermented (Fig. 7).

The biochemical analysis of the fermented samples showed the highest total acidity of 15.36 μmol/mL in a mixed culture-fermented sample on day 3 of the fermentation while the lowest value of 0.36 μmol/mL was observed in a spontaneously fermented sample. During the fermentation of the tested cereals pH fell from (5.75-6.1) to 3.2±0.6 in all the slurries. There was decrease in the pH values of the various “OGI” samples as the fermentation progressed (Fig. 8 and 9).

**DISCUSSION**

The health effects exerted by LAB include production of lactic acid and minor amounts of acetic and formic acids. This causes a drop in pH and thereby growth inhibition of food spoilage organisms, killing of certain pathogens, production of antimicrobial compounds and probiotic effects which are all common features of LAB.

In the present study, organic acid producing capacity and antimicrobial properties of *L. pentosus* and *L. acidophilus* used as starter cultures during fermentation
of cereals for “OGI” production were investigated. It was discovered that the titratable acidity of the cereals fermented with mixed culture has more than 50% increase over the control and each strain used individually. Production of organic acid by *Lactobacillus* is considered primary regulator of microbial activity in the environment of the product. The very low pH of the environment prevents thriving of acidosensitive pathogens, which cannot therefore exert their effect.

Lactic fermentation has been shown to lower the levels of proteinase inhibitors in cereal porridges thereby increasing the availability of essential amino acids such as lysine, leucine, isoleucine, methionine and even tryptophan, and consequently improving the protein quality of cereal grains (Holzapfel, 2002).

This study revealed that the *Lactobacillus*-fermented cereal gruels yielded detectable to significant levels of lysine and arginine coupled with higher level of isoleucine when compared with locally fermented gruels and the maize kernels irrespective of soil conditions, in which they were grown, their genetic background and cotyledon architecture (IITA, 1982). Maize and sorghum are cereals poor in lysine (Chavan and Kadam, 1989).

The detection of these essential amino acids in the fermented gruels is an indication of nutritional improvement and empowerment by fermentation. Kazanas and Fields (1981), reported that natural lactic fermentation of ground grain sorghum produced significant increases (p<0.001) in available lysine/leucine, isoleucine and methionine. Adebawo et al. (2000), had previously reported higher lysine content in ‘OGI’ produced by genetically engineered *L. plantarum*. Mbata et al. (2006) reported fermented cereal gruels with higher lysine, arginine and methionine contents under laboratory adapted conditions. Shekib (1994) reported that lysine content increased significantly in fermented rice even higher than that of FAO/WHO pattern. Lysine and arginine are essential growth factors in foods for growing children and their non-availability has been associated with low-growth for age and stunting in malnourished children. Physiologically, lysine contributes to the active sites of enzymes and helicity of proteins, while arginine serves as a substrate for nitric oxide and polyamine biosyntheses (Mbata et al., 2006).

*Lactobacillus* acidophilus and *L. pentosus* used in this present study seem to have a very active metabolic pathway that can synthesize diacetyl and acetoin. This might be connected with high production of lactic acid and possibly pyruvate which is a key metabolite involved in the synthesis of diacetyl. Diacetyl production could be a way by which these strains eliminate pyruvate which is highly toxic to cells when they accumulate as the cells enter the stationary phase. Production of diacetyl and consequently acetoin could also be as a result of the metabolism of citrate through an active citrate permease/citrate lyase, and requires an active diacetyl synthetase which will convert acetate to diacetyl. It is presumed that these enzyme systems are active in *L. acidophilus* and *L. pentosus*.

Organoleptic properties were generally superior compared to when neither of the starters were used. The overall acceptability of each of the cereal gruels studied by the panelists was observed to be above 50%. Despite its relatively lower organoleptic values, the overall acceptability of the spontaneously fermented cereal gruels was observed to score above 50%. Poverty has been found to further constrain access to food in most developing countries including Nigeria. It has also been found to modulate the perception of consumers on taste, smell, aroma and acceptability (Mbata et al., 2006).

The ability to degrade available protein in a medium to assimilable amino acids and peptides is essential in the production of fermentation products and implies the necessity of proteolytic system in any organism used as starter culture in fermentation process. The degradation and utilization of protein by the starter will usually require the concerted action of proteinases, peptidases, and amino acid and peptide uptake system. This system is believed to be brought into use by these two starter strains and even further enhanced when the two are used in combination.

**CONCLUSION**

The use of these strains of *Lactobacillus* species as starter culture in the fermentation of cereal gruel resulted in a product of increased nutritive value, acceptable organoleptic characteristics, and flavour properties.

**Significance and Impact of study:** This shows that the readily affordable traditionally weaning infant food can be biologically improved into an acceptable protein rich food that is safe for human consumption. This will reduce significantly protein deficiency associated with infantile diarrhoea in our environment.

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